09/652,493

WEST

Generate Collection Print

L3: Entry 9 of 14 File: USPT Aug 28, 2001

DOCUMENT-IDENTIFIER: US 6280931 B1
TITLE: Method for specifically amplifying a dystroglycan, .alpha.-sarcoglycan, or endothelin Breceptor cDNA of an extremely small

Brief Summary Text (5):
Inherited diseases, cancers and some types of progeria and dementia are caused by occurrence of variation in specific genes. Thus, these diseases are often called generically "genetic diseases". In these genetic diseases, abnormal characters appear only in such an organ or tissue where a specific gene involved in the disease is expressed. However, the variation in the gene itself is present in any tissue in the body. Therefore, as a technique for diagnosing a genetic disease, a method may be considered which comprises extracting genomic DNA from leukocytes in peripheral blood that is most easy to sample and then amplifying the genomic DNA.

Brief Summary Text (21):
As examples of the cDNA, a cDNA encoding dystroglycan, .alpha.-sarcoglycan or endothelin B receptor may be given.

Drawing Description Text (4):
FIG. 3 is a photograph showing the results of agarose gel electrophoresis for dystroglycan.

Detailed Description Text (5):
In the present invention, those mRNAs which are expressed tissue-specifically and are difficult to isolate from the tissue by biopsy or the like may be enumerated particularly as a target mRNA for amplification. Such mRNAs are reverse-transcribed to cDNAs and then amplified. Specific examples of such cDNAs include a cDNA encoding human dystroglycan (hDG) involved in progressive muscular dystrophy, a cDNA encoding human .alpha.-sarcoglycan (h.alpha.-SG) involved in another progressive muscular dystrophy, and a cDNA encoding human endothelin B receptor (hET.sub.B) involved in Hirschsprung's disease. Since the above proteins are expressed in specific tissues (e.g., hDG and hET.sub.B are expressed in brain and heart), the mRNAs encoding those proteins leak out into peripheral blood leukocytes in only extremely small quantities. Therefore, the method of amplification of the present invention is particularly useful in amplifying such mRNAs. However, the target of amplification of the present invention is not limited to the mRNAs and cDNAs described above.

Detailed Description Text (28):
Amplification of a cDNA Encoding Human Dystroglycan (hDG) which is One of the Candidate Proteins Involved in Progressive Muscular Dystrophy

Detailed Description Text (29):
In this Example, cDNA from peripheral blood was amplified for the purpose of amplifying a cDNA encoding human dystroglycan (hDG) which is one of the candidate proteins involved in progressive muscular dystrophy.

Detailed Description Text (35):
The primer hDGP (SEQ ID NO. 1) for cDNA synthesis corresponds to positions 3202 to 3226 (located 123 bp downstream of the coding region) of the cDNA sequence shown in SEQ ID NO. 10 encoding dystroglycan (hDG) involved in progressive muscular dystrophy.

Detailed Description Text (41):
The 5' primer corresponds to positions 294 to 324 (located 71 bp upstream of the coding region) of the cDNA sequence shown in SEQ ID NO. 10 encoding human dystroglycan (hDG) involved in progressive myodystrophy, and the 3' primer corresponds to positions 3164 to 3194 (located 85 bp downstream of the coding region) of the same cDNA sequence.

Detailed Description Text (68):
According to the present invention, it is possible to amplify a cDNA encoding the entire coding region of a target mRNA

expressed only in an extremely small amount by performing one PCR. Accordingly, with the present invention, it is possible to perform a practical and rapid screening of a candidate causative gene for various inherited diseases or cancers.

Other Reference Publication (9):
Nelson (1996) Cancer Research vol. 56 p 663-668 (1996, Feb. 15).*

CLAIMS:

1. A method for detecting a tissue specific cDNA having a coding region which is not actively transcribed in peripheral blood comprising:

synthesizing the tissue specific cDNA from a target mRNA in the peripheral blood using an oligonucleotide primer shown in SEQ ID NO: 1, wherein the tissue specific cDNA encodes dystroglycan and has a base sequence shown in SEQ ID NO: 10 or a fragment thereof;

amplifying the coding region of the resultant cDNA by only one round of PCR using a 5' primer and a 3' primer each having 20-40 bases, wherein the 3' primer is located between the coding region and the oligonucleotide primer; and

detecting the amplified cDNA.

10. A kit for detecting a cDNA encoding dystroglycan in peripheral blood comprising a set of oligonucleotide primers having the base sequence shown in SEQ ID NO:1 for cDNA synthesis and SEQ ID NOS:2 and 3 for PCR.